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Real-time polymerase chain reaction for the quantitative detection of soybean in meat products

Mafra, Isabel (1,2); S. Amaral, Joana (1,3); Soares, Sónia (1,3); P.P.Oliveira, M. Beatriz (1)

1: REQUIMTE, Serviço de Bromatologia, Faculdade de Farmácia, Universidade do Porto, Portugal;

2: CBQF/Escola Superior de Biotecnologia UCP, Portugal;

3: ESTiG, Instituto Politécnico de Bragança, Campus de Sta. Apolónia, 5301-857 Bragança, Portugal

E-mail: isabel.mafra@ff.up.pt

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Abstract

Soybean protein is reported to be the most widely used vegetable protein in the meat industry. Several characteristics of soybean protein such as the emulsifier properties, preventing the coalescence of fat during heating, and the increased capacity of water-holding improving the texture of the final product are reasons for its generalised use. However, as soybean is included in the group of ingredients potentially allergenic, if not declared, it can be considered a hidden allergen, representing a potential risk to sensitised individuals. Various methods have been proposed for the detection of soybean in food products, mainly based on the analysis of proteins, such as immunological assays, electrophoretic and chromatographic methods. Due to the higher stability of nucleic acids when compared to proteins and to their ubiquity in every type of cells, DNA molecules have been the target compounds for species identification in several recent works [1].

The aim of this work was to develop highly sensitive and fast DNA-based techniques as alternative to the currently used protein-based methods. For that purpose, binary mixtures of soybean protein in pork's meat were prepared. In a previous stage of this project, qualitative PCR techniques were successfully applied in the species-specific PCR detection of soybean lectin gene in Frankfurt type sausages [2]. In the present work, we propose a novel approach for the quantitative detection of soybean in processed meat products by means of real-time PCR coupled with fluorescent TaqMan probes. The assays involved the amplification targeting an eukaryotic DNA fragment with specific primers and probe as reference gene for quantification. The amplification of soybean lectin gene was performed in parallel reactions using specific primers and probe. With the values of cycle threshold (Ct) a calibration standard curve was obtained using the DDCT method, allowing the detection and quantification of soybean protein in pork's meat in the proportions of 0.1% to 50%. The established real-time PCR technique was successfully applied in the confirmation of qualitative PCR results and in the estimation of soybean protein in commercial meat products.

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